Early reports

Parkinson's disease, pesticides, and glutathi n transferase polymorphisms

Alessandra Menegon, Philip G Board, Anneke C Blackburn, George D Mellick, David G Le Couteur

Summary

Background Parkinson's disease is thought to be secondary to the presence of neurotoxins, and pesticides have been implicated as possible causative agents. Glutathione transferases (GST) metabolise xenobiotics, including pesticides. Therefore, we investigated the role of GST polymorphisms in the pathogenesis of idiopathic Parkinson's disease.

Methods We genotyped by PCR polymorphisms in four GST classes (GSTM1, GSTT1, GSTP1, and GSTZ1) in 95 Parkinson's disease patients and 95 controls. We asked all patients for information about pesticide exposure.

Findings The distribution of the GSTP1 genotypes differed significantly between patients and controls who had been exposed to pesticides (controls *vs* patients: AA 14 [54%] of 26 *vs* seven [18%] of 39; AB 11 [42%] of 26 *vs* 22 [56%] of 39; BB 1 [4%] of 26 *vs* six [15%] of 39; AC 0 *vs* four [10%] of 39, p=0·009). No association was found with any of the other GST polymorphisms. Pesticide exposure and a positive family history were risk factors for Parkinson's disease.

Interpretation GSTP1-1, which is expressed in the blood-brain barrier, may influence response to neurotoxins and explain the susceptibility of some people to the parkinsonism-inducing effects of pesticides.

Lancet 1998; **352:** 1344–46

Departments of Pharmacology and Medicine, University of Sydney, Canberra Clinical School, The Canberra Hospital, Canberra, Australia (A Menegon MSc, D G Le Couteur FRACP); **Molecular Genetics Group, John Curtin School of Medical Research, ANU, ACT 2601, Australia** (A Menegon, Prof P G Board PhD, A C Blackburn PhD); **and Department of Medicine, University of Queensland, Princess Alexandra Hospital, Woolloongabba** (G D Mellick PhD)

Correspondence to: Prof Philip G Board

Introduction

Many studies suggest that Parkinson's disease is more common among people who report exposure to pesticides.[1,2] There are case reports of acute parkinsonism after exposure to paraquat[3] and organophosphate insecticides.[4] Not all people exposed to pesticides, however, develop Parkinson's disease. Reports suggest that some people may have a genetic susceptibility to Parkinson's disease mediated by enzymes involved in the disposition of pesticides and other putative neurotoxins, for example, cytochrome P450 2D6 and N-acetyltransferase-2.[5,6]

Glutathione transferases (GSTs) are a ubiquitous group of detoxification enzymes involved in the metabolism of pesticides[7] and other toxins.[8] The activity of GST has been reported to be normal in the brains of people with Parkinson's disease.[9] Only studies of single substrates have been reported, and in human beings there are several classes of GST with different substrate specificities and tissue distributions.[10] Several GST polymorphisms have been identified.[11–15] The polymorphisms of the GSTM1 and GSTT1 loci arise from the complete deletion of each gene[15,16] and can affect substantially the metabolism of some substances, such as trans-stilbene oxide (GSTM1)[16] and the alkylhalides (GSTT1).[15] The polymorphisms at the GSTP1 and GSTZ1 loci result in aminoacid substitutions that have more subtle effects. There is good evidence that the polymorphisms of GSTP1 effect substrate selectivity and stability.[17,18]

These polymorphisms and their effects on substrate selectivity make the GSTs plausible candidate genes for susceptibility to Parkinson's disease. We investigated the association between Parkinson's disease pesticide exposure and polymorphisms in four GST genes.

Methods

Patients

We recruited 96 patients with Parkinson's disease from community groups, hospital wards, and outpatient clinics throughout south-east Queensland and central west New South Wales, Australia.[3] All patients were white. We used the criteria of Calne and colleagues[19] to diagnose Parkinsons disease. We also recruited 95 healthy controls. We collected demographic data and information about possible risk factors by structured questionnaire. Pesticide exposure was defined as more than once-weekly exposure for more than 6 months before the onset of disease and, when possible, we recorded details such as the type of pesticide, duration of exposure, and whether exposure was through farming, industrial exposure, gardening, or other mechanisms. For family history of Parkinson's disease, we included first-degree and second-degree affected relatives. All participants gave informed consent. The study was approved by the Princess Alexandra Hospital ethics committee.

Polymorphism	Patients (n=95)	Controls (n=95)
T1 deletion		
Positive	79	71
Negative	16	24
M1 deletion		
Positive	46	47
Negative	49	48
Z1 Lys32Glu		
AA	10	6
AG	36	42
GG	49	47
A/G ratio	56/134	54/136
Z1 Arg42Gly		
AA	1	0
AG	8	13
GG	86	82
A/G ratio	10/180	13/177
P1		
AA	33	39
AB	42	37
AC	12	9
BB	8	7
BC	0	3
A/B/C ratio	120/58/12	124/54/12

Table 1: **Distribution of alleles and genotypes of GST polymorphisms**

Methods

We collected venous blood samples into tubes containing edetic acid. We extracted genomic DNA from leucocytes. GSTM1 deletion was detected by PCR with the primers 5'CTGCCCTACTTGATTGATGGG3' and 5'CTGGATTGTAGCAGATCATGC3' and the product analysed by 1·5% agarose gel electrophoresis.[14] We detected the GSTT1 deletion by PCR with the primers 5'TTCCTTACTGGTCCTCACATCTC3' and 5'TCACCGGATCATGGCCAGCA3' and analysed the product by 1·5% agarose gel electrophoresis. The GSTZ1 Lys32Glu polymorphism is caused by an A→G transition. We detected this transition by PCR with the primers 5'TGACCACCCAGAAGTGTTAG3' and 5'AGTCCACAAGACACAGGTTC3', digestion of the product with *Alw*26 I, and analysis of the digestion products by 12% polyacrylamide gel electrophoresis.[13] The GSTZ1 Arg42Gly polymorphism is caused by an A→G transition. We detected this transition by PCR with the primers 5'TGACCACCCAGAAGTGTTAG3' and 5'AGTCCACAAGACACAGGTTC3', digestion of the product with *Fok* I, and analysis of the digestion products by 12% polyacrylamide gel electrophresis.

The GSTP1 gene variants are caused by base-pair transitions at nucleotides +313 and +341.[15,20,21] GSTP1*A has isoleucine at position 105 and alanine at position 114. GSTP1*B has valine at position 105 caused by an A→G transition at nucleotide +313. GSTP1*C has valine at position 105 and also valine at position 114 caused by a C→T transition at nucleotide +341.[20] We detected the polymorphism at nucleotide +313 by PCR with the primers 5'CTCTATGGGAAGGACCAGCAGGAG3' and 5'CAAGCCACCTGAGGGGTAAGG3', digestion of the product with *Alw*26 I, and analysis of the digestion products by 12% polyacrylamide gel electrophoresis. The polymorphism at nucleotide +341 was detected by PCR with the primers 5'GTTGTGGGGAGCAAGCAGAGG3' and 5'CACAATGAAGGTCTTGCCTCCC3', digestion of the product with *Aci* I, and analysis of the digestion products by 12% polyacrylamide gel electrophoresis.

	Genotype frequency (number of patients)			
	AA	AB	BB	AC
Controls (n=26)	0·54 (14)	0·42 (11)	0·04 (1)	0 (0)
Patients (n=39)	0·18 (7)	0·56 (22)	0·15 (6)	0·10 (4)

Table 2: **Distribution of GSTP1-1 polymorphisms in participants exposed to pesticides**

Statistical analysis

We tested for differences in distribution of the genotypes between patients and controls with the χ^2 test. We analysed the variables age, sex, family history of Parkinson's disease, and pesticide exposure with logistic regression.

Results

The mean age of the patients (59 men, 39 women) was 72 (SD 9) years and of the controls (73 men, 22 women) was 67 (12) years. Logistic-regression analysis for age, sex, family history, and pesticide exposure showed that family history (odds ratio 4·2 [95% CI 1·3–14], p=0·02) and pesticide exposure (2·3 [1·2–4·4], p=0·02) were significant risk factors. The distribution of genotypes for any of the polymorphisms did not differ significantly (table 1).

Because GSTs are involved in the detoxication of xenobiotics, we analysed only participants who reported exposure to pesticides (39 patients, 26 controls). The distribution of the genotypes of the GSTP1 polymorphism differed significantly between patients and controls (p=0·009, table 2). These differences seemed to be secondary to an excess of heterozygotes and non-carriers of A alleles among patients. These results remained significant after Bonferroni correction for five comparisons. There were no significant differences in the distributions of any of the other GST polymorphisms.

Discussion

GSTs have direct antioxidant activity[22] and are involved in the metabolism of dopamine.[8] Most positive gene studies in Parkinson's disease have been those that have studied genes involved in the disposition of toxins, such as CYP2D6,[5] NAT2,[6] and DAT1.[23] Mutations in the α-synuclein gene have also proved to cause some cases of familial Parkinson's disease.[24] The association with Parkinson's disease of polymorphisms in several genes suggests a heterogeneous genetic aetiology, similar to Alzheimer's disease.

There have been only a few studies of GST polymorphisms and Parkinson's disease. No association has been identified with the GSTM1 and GSTT1 deletion polymorphisms.[6,25] Similarly, we were unable to detect any association between idiopathic Parkinson's disease and the GSTM1 or GSTT1 deletion polymorphisms, or with polymorphisms in GSTP1 or GSTZ1.

When analysis was restricted, however, to participants who reported exposure to pesticides, significantly more patients with Parkinson's disease had GSTP1 gene variants. The GSTP1 polymorphism was first reported by Board and colleagues[11] and subsequent studies showed important allelic differences in substrate selectivity.[17,18,20] Our results are preliminary and are limited by the selection bias and case ascertainment bias inherent in case-control studies, and also by recall bias. The odds ratios for family history and pesticide exposure are, however, similar to those reported elsewhere. We confined the analysis to participants with pesticide exposure because genes involved in toxin metabolism will determine disease pathogenesis only if toxin exposure has occurred.

There are several possible explanations for the association of GSTP1 gene variants and Parkinson's disease in patients who have been exposed to pesticides. First, the association may be secondary to genetic

linkage to other genes near the GSTP1 locus. GSTP1 is located on chromosome 11q13,[11] and genes of interest in this region include aldehyde dehydrogenase. GSTP1 is, however, involved directly in pesticide metabolism.[26] In the rat, π class GST (GST Y_p) is present in high concentration in the brainstem,[27] and in human beings GSTP1-1 is present in the brain and blood-brain barrier.[28] These findings make it likely that GSTP1 is the susceptibility gene rather than a linkage marker.

A probable explanation for the observed association is that the polymorphisms influence the ability of GSTP1-1 to detoxify pesticides that may have neurotoxic effects. Possession of the A allele seems to be protective. GSTs metabolise various pesticides, many of which are lipophilic electrophiles.[7] GSTP1 polymorphisms confer differential susceptibility to cancers of the bladder, testicle, prostate,[29] and brain[20] presumably because of their various capacities to detoxify carcinogens.[17,18,20] The effect of GSTP1 polymorphisms on the ability to metabolise pesticides has not been reported. However, mutations at codons 47 and 101 influence the inactivation of GSTP1-1 by the pesticides captan and captofol.[26] Similarly, the association of polymorphisms in the genes for cytochrome P450 2D6 and N-acetyltransferase with Parkinson's disease are thought to be mediated by altered xenobiotic metabolism.[5,6]

The GSTP1 polymorphisms may also increase the conversion of a pesticide to a toxic metabolite. Previous studies have shown that GST enzymes can increase the toxicity of some substrates.[30] This hypothesis might be supported by the observation that patients who are heterozygous at the GSTP1 locus seem to have increased susceptibility to Parkinson's disease (table 2) because heterozygosity would be expected to increase the range of substrates metabolised by the GSTP-1 enzyme.

The significant differences in GSTP1 genotype frequencies we have shown in patients with Parkinson's disease exposed to pesticides might explain susceptibility to Parkinson's disease after pesticide exposure.

Contributors

Alessandra Menegon did the genotyping. Philip Board was involved with the design and supervision of the study, technique development, and data analysis. Anneke Blackburn developed the PCR technique. George Mellick isolated the DNA. David Le Couteur recruited patients, designed and supervised the study, and did the statistical analysis.

Acknowledgments

This study was supported by the National Health and Medical Research Council of Australia and the Geriatric Medical Foundation of Queensland. We thank Marj Coggan and Sally McCann for technical assistance, and Susan Pond for her support.

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